Evaluation of Colour Content in Grapes Originating from South Moravia

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Abstract: The content of total anthocyanins was determined in grapes of nine grapevine (*Vitis vinifera* L.) varieties belonging to the traditional blue vine varieties grown in south Moravia (Czech Republic) within the period of 2002–2007. Factors of vintage and health condition of grapes were observed. The material balance of the colour substances in grapes was related to the dry matter of skins of berries and that of fresh grapes used as raw material for vinification. The highest content of pigments was found in the variety Neronet (2.15–4.49 g/kg of fresh grapes), which belongs to the so-called Teinturier varieties. Besides the variety Neronet (containing 30.6–73.4 mg/g of dry skin), high contents of total anthocyanins in dry skins were found in the varieties Dornfelder (24.7–59.0 mg/g of dry skin) and Cabernet Moravia (20.1–59.3 mg/g of dry skin). In the long run, the lowest concentrations of pigments were determined in grapes of the varieties Blauer Portugieser (0.51–1.02 g/kg of fresh grapes) and Pinot noir (0.27–0.60 g/kg of fresh grapes). The highest colour capacity was found in grapes harvested in the 2003 and 2006. Contents of anthocyanins were significantly lower (P = 0.001) in damaged grapes. Grapes containing more than 40% of rotten berries revealed in skins and whole grapes only 41% and 55%, respectively, of the colour capacity of the healthy raw material.

Keywords: total anthocyanins; extraction; grape; grape berries; skin of grape berries; grape varieties; rotten grapes

In the genus *Vitis*, the main cause of colouration of grape berries is the accumulation of anthocyanins in the skin and, in some cases, also in the pulp, i.e. in the so-called Teinturiers; the intensity of this colouration changing form light red to deep blue hue. The profile and concentration of anthocyanins in grapes show an essential effect on both the colour parameters of the red wines produced and the changes in their colour. The applied methods of vinification and wine stabilisation as well as the conditions of wine ageing modify the contents of anthocyanins in red and/or rosé wines and, at the same time, also their colour intensity and hue (Gao *et al.* 1997; Bautista-Ortin *et al.* 2005; Romero-Cascales *et al.* 2005; Castillo-Sanchez *et al.* 2006; Gonzalez-Neves *et al.* 2008, and others).

In the course of ripening, the accumulation of pigments in grapes is characterised initially by a slow increase in their contents which is followed by a steep increase and by subsequent stabilisation; towards the end of the physiological ripeness of grapes, the contents of anthocyanins can even decrease (Pirie & Mullins 1980; González-Sanjosé & Diez 1992; Keller & Hrazdina 1998, and others). The total amount of the accumulated anthocyanin pigments in grapes is strongly variety-dependent; the problems associated with their accumulation were studied by many authors (Fernandez-Lopéz *et al.* 1992; Balík 1994; Fournand *et al.* 2006; Pérez-Magariño *et al.* 2006; González-Neves *et al.* 2007; Guidoni *et al.* 2008; Navarro *et al.* 2008, and others).

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Mazza (1995) published a survey of all anthocyanins occurring in fresh grape berries of some grapevine (*Vitis vinifera* L.) cultivars, e.g. Cabernet Sauvignon (0.86–0.98 g/kg), Gamay (0.27–0.59 g/kg) and Pinot noir (0.33 g/kg). The Teinturier variety Alicante-Bouchet contained 5.2 g/kg of anthocyanins; with a proportion of 78.4% being present in the skin. The contents of these pigments in the pulp, stalks, and seeds were 18.7%, 1.6%, and 1.3%, respectively. As results from the literary data, varieties Shiraz and Cabernet Sauvignon also show a high colour potential (2.2 and 1.7 g/kg of grapes, respectively). Lower concentrations were reported in the varieties Monastrell and Pinot noir with their average contents of anthocyanins of 1.139 and 0.825 g/kg, respectively (Lemperle *et al.* 1973; Bourzeix & Saquet 1975; Fernandéz-Lopéz *et al.* 1992).

In the same varieties, the published differences in of colour capacity were influenced mostly by differences in geographical conditions, climatic characteristics of individual years, intensity of agronomical measures practises and, possibly, also clones (Crippen & Morrison 1986; Ojeda *et al.* 2002; Spayd *et al.* 2002; Downey *et al.* 2006; Yamane *et al.* 2006; Guidoni *et al.* 2008, and others). The differences in the methods of extraction anthocyanins from the skin, as used by different authors, may be another source of the variability in the colour capacity of grapes (Drdák *et al.* 1991; Guidoni *et al.* 2002; Ju & Howard 2003; Fournand *et al.* 2006). Several procedures enabling the extraction of grape anthocyanins were compared by Revilla *et al.* (1998). The published results showed that the use of solvents containing up to 1% of 12N hydrochloric acid for the extraction of grape anthocyanins resulted in partial hydrolysis of malvidin-3-*O*-acetylglucoside. This process caused important changes in the relative content of anthocyanins in these extracts, despite its higher efficiency in extracting total anthocyanins in some cases.

The objective of this paper was to compare total content of anthocyanin pigments in grapes of cultivars grown traditionally in south Moravia (Czech Republic) with regard to the year and health condition state of grapes.

MATERIAL AND METHODS

Material and preparation of samples. The content of total anthocyanins in grapes were determined in nine varieties of grapevine (*Vitis vinifera* L.): An – André (= Blaufrankisch × Saint Laurent); CM – Cabernet Moravia (= Zweigeltrebe × Cabernet Franc); Do – Dornfelder = (Helfensteiner × Heroldrebe); Fr – Frankovka (Blaufrankisch); MP– Modrý Portugal (= Blauer Portugieser); Nt – Neronet = (Saint Laurent × Blauer Portugieser) × (Alicante Bouchet × Cabernet Sauvignon); RM – Rulandské modré (= Pinot noir); Sv – Svatovavřinecké (= Saint Laurent) and Zw – Zweigeltrebe. The analyses were performed over a period of six subsequent years (2002–2007) using grapes originating from the vine growing region of Mikulov (south Moravia). There were evaluated and compared grapes sampled in the stage of technological ripeness reached in the individual years and also those with more than 40% of rotten grape berries. Until the analysis, the grapes (altogether 10 pcs of bunch per a cultivar) were stored at the temperature of –18°C for a maximum period of 3 weeks.

Extraction methods. Anthocyanins were extracted from the grapes using a modified method described by Drdák *et al.* (1991). From each grape, altogether 10–15 g of berries were extracted by discontinuous homogenisation in a mixer containing 100 ml of acidified methanol (0.1% HCl in methanol). The skins of the berries coming from the analysed grape were separated manually from the pulp and used for the determination of skin dry matter and for the extraction of anthocyanins. The skin samples (2–3 g) were repeatedly extracted in the Potter-Elvehjem homogeniser containing 100 ml of acidified methanol (0.1% HCl in methanol). Anthocyanins were determined in the centrifuged extracts.

Determination of total anthocyanins. The content of total anthocyanins was estimated by a spectrophotometric method elaborated by Fuleki and Francis (1968); the method is based on the measurement of differences in the absorbance values of anthocyanin pigments extracted in buffers of pH 1 and of pH 4.5, respectively, at 520 nm (spectrophotometer Heloisβ/Unicam). The concentration of total anthocyanins was expressed on the basis of the content of malvidin-3-monoglucoside using the values of molar absorptivity (28 000 l per mol.cm) and molecular weight (562.5 g/mol) (Fernandéz-Lopéz *et al.* 1992). The results obtained were converted to calculate per dry matter of the skins and also to per fresh bunch of grapes.

Determination of sugar content in grape berry and in skin dry matter. The contents of sugars in grape berry (expressed as soluble dry matter of grape must) were determined with the standard Abbe refractometer at 20°C and expressed as weight percentage of sucrose. The dry matter of skin was determined gravimetrically by drying 2–3 g of skins at 105°C to a constant weight.

Reagents and solvents. Buffer pH 1.0 – 0.2 mol/l KCl:0.2 mol/l HCl (25:67); buffer pH 4.5 – 1.0 mol/l sodium acetate:1.0 mol/l HCl:deionised water (100:60:90). Methanol and other reagents were obtained from Fluka (Sigma-Aldrich, Prague, Czech Republic).

Statistical methods. The data were evaluated using the analysis of variance with the Tukey test of the program Statgraphics. The presented values are means of ten measurements.

RESULTS AND DISCUSSION

The material balance of the pigment contents in grapes was related to the dry skin and to the weight of fresh grapes, which were used as raw material for vinification. The content of total anthocyanins found in nine different blue grapevine (*Vitis vinifera* L.) varieties traditionally grown in south Moravia are presented in Table 1. The data obtained in 2002–2007 are supplemented with the values of soluble dry matter of grapes, which expresses the stage of their ripeness at the moment of sampling. Besides the evaluation of healthy

Table 1. Contant of total anthocyanins and soluble dry matter in health grapes of nine varieties depending on vintage and in rotten grapes (n = 10)

Variety		2002	2003	2004	2005	2006	2007	Rot damage
	Ant1 (S.D.)	45.5 (6.2)	57.3 (7.2)	28.4 (5.5)	35.4 (6.1)	46.6 (5.9)	31.9 (7.3)	16.9 (10.4)
An	Ant2 (S.D.)	1.12 (0.19)	1.29 (0.31)	0.91 (0.28)	1.12 (0.18)	1.15 (0.22)	1.02 (0.30)	0.69 (0.45)
	SDM (S.D.)	18.4 (1.2)	22.0 (1.5)	17.4 (1.0)	18.4 (1.2)	19.7 (1.3)	18.0 (1.4)	18.1 (1.7)
	Ant1 (S.D.)	50.1 (7.9)	45.6 (6.9)	49.9 (8.2)	50.8 (7.2)	59.3 (5.8)	48.2 (7.3)	20.1 (11.1)
CM	Ant2 (S.D.)	1.87 (0.28)	1.64 (0.38)	1.76 (0.30)	2.01 (0.30)	2.10 (0.42)	1.71 (0.31)	0.84 (0.48)
	SDM (S.D.)	18.0 (0.8)	19.4 (1.2)	15.4 (1.1)	18.0 (1.2)	19.1 (1.4)	17.0 (1.5)	18.9 (1.3)
	Ant1 (S.D.)	54.6 (6.1)	68.5 (8.5)	48.4 (7.8)	55.0 (6.6)	59.0 (7.0)	50.8 (9.1)	24.7 (10.0)
Do	Ant2 (S.D.)	2.36 (0.31)	2.67 (0.22)	1.97 (0.37)	2.23 (0.37)	2.47 (0.30)	2.04 (0.28)	1.00 (0.45)
	SDM (S.D.)	18.9 (1.1)	20.2 (1.7)	17.0 (1.3)	18.7 (1.3)	20.3 (1.0)	17.7 (1.2)	20.1 (1.5)
	Ant1 (S.D.)	45.1 (8.0)	46.5 (6.8)	33.2 (5.9)	41.9 (6.9)	47.1 (8.2)	38.0 (9.2)	11.1 (9.6)
Fr	Ant2 (S.D.)	0.87 (0.15)	0.87 (0.21)	0.78 (0.18)	0.81 (0.18)	1.13 (0.33)	0.73 (0.26)	0.60 (0.40)
	SDM (S.D.)	18.7 (1.2)	20.1 (1.2)	16.4 (1.0)	18.1 (0.9)	19.5 (1.5)	17.5 (1.1)	18.5 (1.4)
	Ant1 (S.D.)	26.9 (5.2)	30.2 (5.7)	16.2 (7.2)	22.9 (6.5)	20.9 (6.4)	34.6 (7.0)	12.4 (6.3)
MP	Ant2 (S.D.)	0.51 (0.19)	0.56 (0.34)	0.55 (0.36)	0.84 (0.14)	1.02 (0.22)	0.79 (0.28)	0.52 (0.36)
	SDM (S.D.)	17.4 (1.6)	18.0 (1.3)	15.8 (1.2)	17.2 (0.8)	17.9 (1.7)	16.7 (1.2)	18.0 (1.3)
	Ant1 (S.D.)	65.0 (8.4)	69.0 (8.9)	52.0 (6.0)	59.0 (7.7)	73.4 (7.4)	62.5 (9.1)	30.6 (12.8)
Ne	Ant2 (S.D.)	3.89 (0.38)	4.49 (0.41)	3.24 (0.27)	4.04 (0.30)	4.18 (0.30)	3.77 (0.26)	2.15 (0.51)
	SDM (S.D.)	18.3 (1.0)	19.0 (1.2)	15.9 (1.0)	16.9 (1.4)	18.8 (1.5)	17.4 (1.4)	18.1 (1.3)
	Ant1 (S.D.)	21.0 (5.6)	27.0 (4.2)	12.0 (4.9)	20.0 (6.7)	30.0 (7.2)	14.0 (6.6)	6.4 (4.0)
RM	Ant2 (S.D.)	0.40 (0.18)	0.60 (0.28)	0.41 (0.15)	0.54 (0.21)	0.59 (0.27)	0.45 (0.21)	0.27 (0.13)
	SDM (S.D.)	20.7 (1.2)	22.1 (0.9)	19.0 (1.7)	20.3 (0.8)	21.3 (1.2)	20.0 (1.3)	21.3 (1.7)
	Ant1 (S.D.)	38.4 (9.2)	47.3 (6.8)	31.8 (5.9)	42.1 (7.0)	40.7 (7.8)	43.6 (8.3)	17.6 (10.1)
Sv	Ant2 (S.D.)	1.71 (0.25)	2.10 (0.33)	1.34 (0.19)	1.87 (0.36)	1.77 (0.18)	1.88 (0.20)	0.75 (0.49)
	SDM (S.D.)	18.7 (1.4)	19.9 (1.1)	16.5 (1.3)	18.5 (1.5)	19.2 (1.1)	18.8 (1.1)	18.3 (1.5)
	Ant1 (S.D.)	45.6 (8.9)	43.5 (6.1)	29.5 (10.0)	40.4 (1.2)	50.5 (8.8)	30.7 (7.5)	17.6 (10.9)
Zw	Ant2 (S.D.)	1.55 (0.35)	1.43 (0.25)	0.93 (0.31)	1.30 (0.30)	1.50 (0.28)	1.23 (0.20)	0.68 (0.45)
	SDM (S.D.)	18.6 (1.0)	19.1 (1.6)	15.5 (1.3)	18.3 (1.2)	19.6 (1.4)	16.7 (1.1)	19.6 (1.5)

For key to varieties, see Materials and Methods; Ant1 = mean of total anthocyanins (mg/g dry skin); Ant2 = mean of total anthocyanins (g/kg fresh grapes); SDM = mean of soluble dry matter (%); (S.D.) = standard deviation grapes Table 1 involves also the data on grapes with more than 40% of rotten berries.

Over the period of six years under study, the highest average content of pigments in the skins of grape berries was found out in the variety Neronet (63.5 mg/g of dry skin), which differed significantly from all other varieties under study (excepting the variety Dornfelder with 56.1 mg/g of dry skin). A high content of anthocyanins in the skins was recorded also with the grapes of the variety Cabernet Moravia (50.6 mg/g of dry skin). Another group comprise the grapes of the varieties of a medium colour capacity, i.e. Zweigeltrebe, Saint Laurent, André, and Blaufrankisch (40.0–42.0 mg/g of dry skin). Lower contents of anthocyanins were observed in the skins of grape berries of the varieties Blauer Portugieser and Pinot noir (25.3 and 20.7 mg/g of dry skin, respectively) (Table 2, Figure 1). Drdák *et al.* (1991) compared the total contents of anthocyanins in the dry skins of blue grape varieties (*Vitis vinifera* L.) and of interspecific hybrids and recorded contents ranging from 12.8 to 105.0 mg/g of dry skin. Out of all varieties of *Vitis vinifera* L. under the study, they found the highest concentration of pigments in the grapes of the variety Neronet (78.4 mg/g dry skin).

The variety Neronet belongs to the so-called Teinturier varieties. For that reason, it was not surprising that its grapes also showed the highest average content of pigments as expressed with regard to the bunch of grapes and differed significantly ($P < 0.05$) from the grapes of all other studied varieties under study (3.93 g/kg of fresh grapes). Even the damaged grapes of the Neronet variety with more than 40% of rotten berries showed a colour capacity comparable with those of other varieties under study (Table 3, Figure 2). Expressed to fresh grapes, higher contents of pigments were determined in the varieties Saint Laurent, Cabernet Moravia, and Dornfelder (i.e. 1.78–2.29 g/kg of fresh grapes). No statistically significant difference in the content of anthocyanins was found out between the grapes of André and Zweigeltrebe varieties (1.10 and 1.32 g/kg of fresh grapes, respectively), their contents of anthocyanins being similar to the value reported for the variety Monastrell (1.14 g/kg of fresh grapes) by Fernández-Lopez *et al.* (1992). The varieties Pinot noir, Blauer Portugieser, and Blaufrankisch showed lower

Table 2. Means of total anthocyanins in the grape skins for six vintages and homogeneous groups of varieties (Tukey, $P = 0.05$)

Variety	Anthocyanins (mg/g dry skin)	Homogeneous groups
RM	20.7	A
MP	25.3	A
Zw	40.0	B
Sv	40.7	B
An	40.9	B
Fr	42.0	B
CM	50.6	BC
Do	56.1	CD
Ne	63.5	D

For key to varieties see Materials and Methods

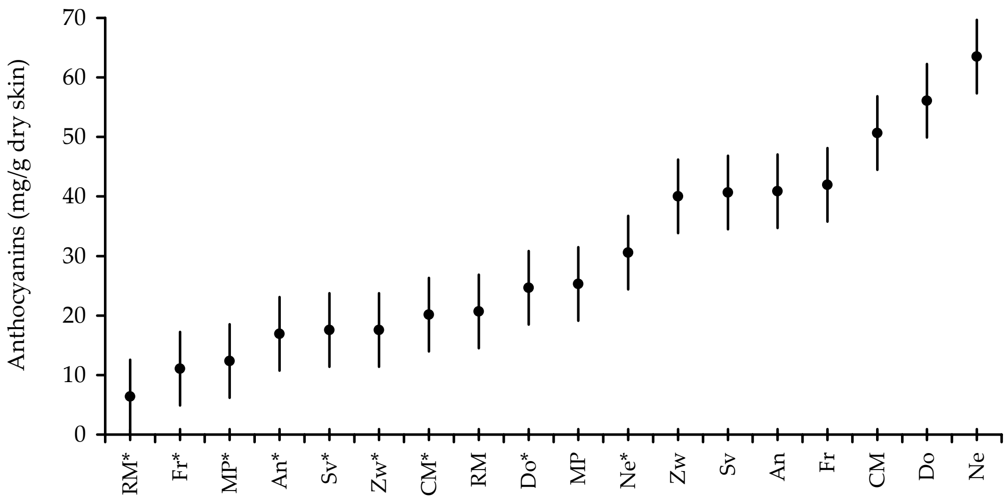

Figure 1. Means of total anthocyanins and Tukey HSD intervals ($P = 0.05$) in the grape skins for six vintages depending on variety and health state (for key to varieties see Materials and Methods; *rot damage grapes)

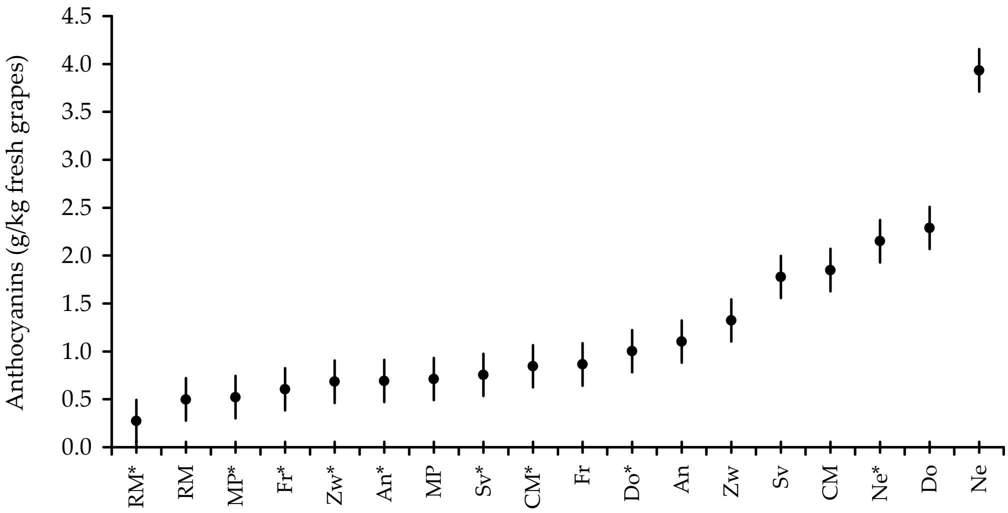

Figure 2. Means of total anthocyanins and Tukey HSD intervals ($P = 0.05$) in the fresh grapes for six vintages depending on variety and health state (for key to varieties, see Materials and Methods; *rot damage grapes)

pigment contents in whole grapes (0.50–0.86 g/kg of fresh grapes). Lamikanra (1989) published a relatively wider range of total anthocyanins content between 0.12 and 10.38 g/kg in the berries of the hybrid grapes of *Vitis rotundifolia* Michx.

In the individual years, the concentrations of anthocyanins in the grapes fluctuated, the climatic conditions being obviously a more important factor than the agronomical practises performed in the course of the grapevine plants growth (Guidoni *et al.* 2008). The colour capacity of the grapes was markedly changed above all by their health conditions. Statistically highly significant differences ($P < 0.01$) in the contents of pigments were observed between the healthy and rotten grapes. The grapes with more than 40% of rotten berries contained in the skins and whole grapes only 41% and 55%, respectively, of the colour capacity of the healthy raw material (Figures 1 and 2). On the other hand, no statistically significant correlations were found between maximum content of pigments and maximum sugar content among all the studied varieties. Nevertheless, in the years with higher average sugar contents in the grapes, their contents of pigments were also increased. The relationships existing between the content of anthocyanins and the ripeness index were studied by many authors, however, the correlation coefficients published are markedly different and fluctuating, mainly due to the fact that the total content of anthocyanins is strongly dependent on both the variety and climatic conditions (Pirie & Mullins 1980; González-Sanjosé & Diez 1992; Keller & Hrazdina 1998).

Table 3. Means of total anthocyanins in the fresh grapes for six vintages and homogeneous groups of varieties (Tukey, $P = 0.05$)

Variety	Anthocyanins (g/kg fresh grapes)	Homogeneous groups
RM	0.50	A
MP	0.71	AB
Fr	0.86	AB
An	1.10	BC
Zw	1.32	C
Sv	1.78	D
CM	1.85	D
Do	2.29	DE
Ne	3.93	F

For key to varieties, see Materials and Methods

Although the analysed healthy grapes of the variety Pinot noir showed permanently higher values of sugar content than other varieties (i.e. 19.0–21.3%), their concentrations of pigments were always lower. Especially as compared with that of the year 2004 (16.5%), higher levels of soluble dry matter were found out in healthy grapes harvested in 2003 and 2006 (20.0 and 19.5%, respectively). This was positively manifested in the concentration of pigments in the skin of grape berries because the differences between the aforementioned years were statistically significant ($P = 0.05$) (Table 1,

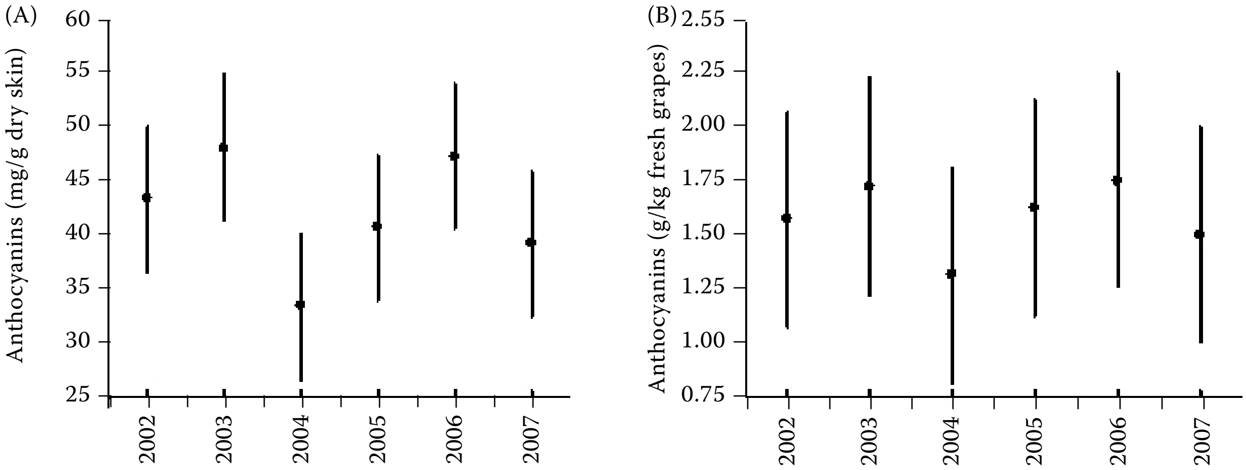

Figure 3. Means of total anthocyanins and Tukey HSD intervals ($P = 0.05$) in the grape skins (A) and the fresh grapes (B) of nine varieties depending on vintage

Figure 3A). Nevertheless, there were no statistically significant differences in the content of pigments in whole grapes; this was due to the fact that the values of colour capacity per the whole apocarpium showed a higher variability (Table 1, Figure 3B).

CONCLUSIONS

The content of total anthocyanins was studied in the grapes of nine grapevine (*Vitis vinifera* L.) varieties grown traditionally in south Moravia over a period of six subsequent years. Content of pigments was strongly dependent on the variety. The conditions existing in the individual vintages modified significantly the degree of ripeness of grapes and their colour capacity. Out of all years under study (2002–2007), the highest colour capacity was determined in 2003 and 2006. The health conditions of the grapes changed substantially their colour capacity. In the skins and whole grapes, the rotten grapes contained only 41% and 55%, respectively, of the colour capacity of the healthy raw material. The highest contents of pigments were permanently observed in the grapes of the varieties Neronet, Dornfelder and Cabernet Moravia.

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